December 20, 1973

Dr. Ray Wu
Division of Biological Sciences
Cornell University
Section of Biochemistry,
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Wing Hall
Ithaca, New York 14850

Dear Dr. Wu:

I am sending separately the strain of SV40 which we have used—small plaque SV40 from strain 776, obtained originally from Ken Takemoto and plaque purified in BSC-1 cells. It is true that strains differ in their Hin digests; in fact, every one of some 6 or so strains we have examined is different. As far as cells are concerned, BSC-1 is O.K., so is CV-1 or vero. In a limited survey we have found no difference in Hin digest of SV40 DNA purified from infected BSC-1, CV-1, or primary AGMK.

To grow a stock SV40, infect with our seed virus at a multiplicity of 0.001 (1 pfu/1000 cells) to minimize growth of defective virus. In BSC-1 complete lysis occurs in about 10 days. Freeze and thaw at this point, shake with chloroform and freeze low speed supernate. To prepare DNA, infect at a multiplicity of 10 to 20 and prepare DNA by Hirt's procedure at ~ 72 hrs (BSC-1) postinfection, when most cells are rounding up but still on the dish. Alternatively, you can let the cells lyse completely and prepare virions. The methods are described or referenced in our papers (or those of many others).

Our BSC-1 cells came originally from Microbiological Associates. Ask for the earliest passage they have. We freeze cells in MEM with 10% fetal calf serum made to 10% glass filtered DMSO. Let them freeze in -20° freezer (to cool slowly) about 4-5 hrs and transfer them to a liquid N2 freezer. Nunc (Vangard) makes a nice 2 ml plastic screw cap tube which is good for this purpose; it must be kept out of the liquid N2, however, i.e., keep them in the N2 gas part of the freezer. We generally use one batch of cells for 3 months or so, passing about once per 10 days, after which we find decreased yield of SV40.

As far as electrophoresis of digests is concerned, we use the apparatus described by Studier in J. Mol. Biol. of this year and conditions given in Danna, Sack and Nathans, J. Mol. Biol. 78, 363, 1973. There is no problem separating C and D on long gel slabs if you run the digest long enough.

Good luck and let me know if I can be of further help.

Sincerely,

Daniel Nathans

DN:as